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# Helophyte impacts on the response of hyporheic invertebrate communities to inundation events in intermittent streams

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**Abstract** The effects of experimental inundation on invertebrate communities in artificial flumes fed with treated wastewater were investigated. Flumes, designed to simulate intermittent river conditions, were planted with three different species of helophytes widely used in river restoration around the water-stressed regions of Europe. Different species of vegetation had different capabilities to reduce the invertebrates negative reaction on inundation, related mostly to rhizosphere density. Of the three helophyte species tested, only one, *Lysimachia vulgaris*, showed significant capabilities to reduce invertebrate negative reaction on inundation. Species richness of invertebrates before and after the inundation did not change in any of the flumes, while species density significantly declined in all flumes except one planted with *L. vulgaris*. This helophyte species was associated with high densities of the Culicidae larvae (common mosquitos) which has severe implications for river restoration and vector management in the region. This study indicates that the selection and establishment of different helophyte species will impact invertebrate communities in restored streams, especially streams experiencing variable inundation conditions.

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## Introduction

Intermittent rivers are defined as those rivers that naturally periodically cease to flow and run dry (Datry et al., 2014). Accord

ing to conservative estimates, about 50% of all the rivers in the world are intermittent (Datry et al., 2014). This percentage is expected to increase with global climate change affecting precipitation regimes around the world (Datry et al., 2014), and increased over-abstraction and hydroengineering activities in perennially flowing rivers.

Invertebrate communities of intermittent rivers represent a patchwork of terrestrial and aquatic taxa (Datry et al. 2014). Communities of intermittent streams have significant overlap with perennial stream communities (Datry et al., 2014). Intermittent streams, however still can have highly specialized communities, as shown by Chou et al. (1999) for the Chironomidae species complexes of the intermittent streams in Kansas. Up to 60% of the all taxa (59 spp) in the investigated streams were either specialist or facultatively-present species (Chou, Ferrington, Hayford, & Smith, 1999). The hydrologic processes that control flow intermittency are also the main determinants of the spatial pattern of terrestrial and aquatic communities. The timing and magnitude of drought and inundation events are the key in structuring intermittent river communities (Larned et al., 2010; Stubbington, 2012; Vander Vorste, Corti, Sagouis, & Datry, 2015). Droughts force aquatic invertebrates to migrate and take refuge in remaining water pools or perennial reaches, or become dormant (Otermin et al. 2002; Stubbington 2012; Vidal-Abarca et al. 2013;). Inundation is also an important factor because it creates an advancing water front (AWF) that can cause a drastic change in community structure (Corti & Datry, 2012) via displacement of terrestrial animals from flooded areas and recolonization of river channels by aquatic fauna from remnant water pools, hyporheic zones, or nearby perennial rivers. The result is that intermittent reaches have higher densities but lower diversities of aquatic invertebrates immediately after passage of an AWF (Corti and Datry 2012). While droughts usually have a gradual onset, inundation of intermittent river beds is a fast process, which has consequentially a larger immediate impact on the structure of the community (Dahm, Baker, Moore, & Thibault, 2003; Lake, 2003). Factors which slow the pace of inundation such as remaining water pools, vegetation and river braiding increase the resilience of the intermittent rivers communities to AWF (Corti & Datry, 2012). As more and more rivers are becoming intermittent such resilience becomes more important (Vidal-Abarca et al., 2013). Understanding how these different buffering aspects help community resilience, including channel vegetation, is key to future management of rivers.

The role of sediment properties and river hydromorphology in controlling the resilience of intermittent river communities to inundation is relatively well studied. In contrast, the influence of other surface and benthic habitat properties, especially the effects of macrophytes, has received considerably less attention (Anderson & Ferrington, 2012; Kail, Brabec, Poppe, & Januschke, 2015). The presence of macrophytes can either amplify or reduce (“*meliorate*”) the effects of hydrological stresses such as flow intermittency on invertebrates (Armitage, Cranston, & Pinder, 1995). On the one hand, plants can create more structured habitats which can shield animals from hydrological extremes (e.g., preventing them from being flushed away by the AWF; (Armitage et al., 1995). On the other hand, extensive growth of the macrophyte roots reduces access to, and the volume of, the hyporheic refugium (Datry et al., 2014). Our knowledge about the influence of AWF on aquatic invertebrates inhabiting the hyporheic refugium of intermittent rivers is still limited despite

recent studies on the structure and function of invertebrate communities in these rivers (Corti & Datry, 2015; Datry et al., 2014; Vander Vorste et al., 2015).

Massive river restoration efforts are underway in many developed countries, especially in the water-stressed regions where there are many intermittent rivers, and aquatic plants are often used in such efforts (Kail et al., 2015). For example, helophytes (semi-submersed aquatic and hydrophilic plants) are frequently used in the Catalonia and other water-stressed regions to stabilize the channels of the intermittent rivers. It is, therefore, important to understand how the application of helophytes in river restoration impacts intermittent river communities and their functioning under hydrological stress, in order to make restorations successful (Kail et al., 2015). A component of this is to find out whether the presence of helophytes makes a difference to the resilience of invertebrate communities in intermittent river systems.

In the present study we tested how semi-submersed macrophytes influence the impact of AWFs on hyporheic invertebrate communities. We conducted an experimental inundation in replicated flumes that included 3 species of helophytes. We hypothesized that: 1) inundation would cause a decrease in the abundance and/or species richness of hyporheic animals since the inundation will flush animals away, and 2) different species of macrophytes would reduce the magnitude of changes in animal abundance after inundation, but to different degrees depending upon belowground vegetation biomass (rhizosphere) conditions. To test these hypotheses we firstly examined the variability of the community structure among flumes planted with different helophytes and how they respond to hydrological changes associated with an inundation event, and secondly tested how different microhabitats created by the rhizospheres of the three helophyte species reduce inundation effects.

## Materials and Methods

### *Experimental set up*

The inundation experiments for this study were conducted at the *Urban River Lab (URL)* experimental research facility in the proximity of Montornés del Vallés, Barcelona, Spain (N 41°54'22'', E 2°23'57''). The URL comprises a set of 18 artificial flumes (60 cm width and 12 m long each) (Fig. 1A). The flumes are fed by effluent of an on-site waste water treatment plant. The experimental design mimics characteristic conditions of intermittent Mediterranean streams, many of which are directly and solely fed by treated wastewater, particularly during the dry season. The flumes have been planted with different species of helophytes. The bottoms of the flumes are covered by 25 centimeter thick layers of coarse (10-20 mm) gravel with some silt occupying about 10% of the total volume. The gravel in the flumes is one that is typically used in restoration projects in the area.

In total, three flumes were sampled for macroinvertebrates: one with *Typha angustifolia*, one with *Lysimachia vulgaris* and one with *Scirpus lacustris*. The sampling campaign took place from June 8-11, 2015. Macroinvertebrates were sampled once a day, along the entire length of the flumes - at the inflow point, and at 1, 3, 5, 7, and 9 meters from inflow (Fig. 1B). At each of these points, small wells were installed to a depth of 15 cm below the gravel surface. Each well consisted of a 60 cm long and 3 cm diameter PVC pipe that was perforated with ten 2 mm holes evenly spread across the lower 5 cm interval of the well.

### *Flume hydrologic conditions*

There was no surface flow in any of the flumes at the start of the experiment except in the sediment-free inflow and outflow basins at the beginning and end of each flume. Thus, at the start of the campaign, we considered the flume systems to be representative of a drying river

where most of the flow and saturated environment is within the hyporheic zone. The mean water residence time in the flumes before the inundation was 8 hours.

Inundation of the upper layers of the sediment was caused by an intensive storm event with peak precipitation between 23:00 h at June 10<sup>th</sup> and 01:00 h at June 11<sup>th</sup>. This storm event also created surface flow in all of the flumes. The inundation created a considerable increase in the water table height of the flumes to a water level of 9 cm above the sediment surface. These conditions are representative of moderate re-wetting events experienced naturally in many intermittent Mediterranean streams. For strategic reasons related to the urgency of the impact of the studied event and to ensure capturing of the immediate reaction to the inundation experiment, no replications of different flume conditions were available for this experiment.

#### *Macroinvertebrates sampling*

Sampling of macroinvertebrates was performed with a Bou-Rouche pump head connected to piezometer tubes (Lencioni, Marziali, & Rossaro, 2008). One liter of unfiltered water was collected with the pump for each sampling point at a pumping rate of 3 L min<sup>-1</sup>. Water samples were filtered through a 250 µm sieve and the retained fraction from the sample was preserved with 76% ethanol on site. In total, 80 samples were collected. Of these, 26 samples could not be analyzed since they were compromised by high organic matter contents, from the WWTP source water and high temperatures on site and during the transportation back to the lab. Taking into account that the URL has presumably low diversity of benthic and hyporheic fauna, due to its high organic pollution, we assume that the 54 samples analyzed, provide a sufficient number to evaluate the reaction of hyporheic invertebrate communities in three types of vegetation (*Lysimanchia*, *Scirpus*, *Typha*) to temporal changes related to the inundation of the flumes. Among those 32 samples were fitting into the selected in the selected time interval (8-11.06.2015), were used for the analysis of the temporal changes.

Macroinvertebrates were determined in the lab using the Leica EZ4 stereomicroscope and Nikon YS100 compound microscope. Organisms were identified to the highest taxonomic resolution possible. Identification was performed on organisms temporarily mounted in glycerol or permanently in Euparal. Voucher material is archived in the School of Geography and Environment, University of Birmingham, UK.

#### *Data analysis*

To test for differences in the communities diversity (S) and abundance (N) along the flumes, based on the sampling point distance from the inflow, ANCOVA was applied with S/ N as the response, distance as covariate and the interaction between distance and plant species to identify longitudinal heterogeneity in the communities, analysis was performed in R version 3.2.4 Revised (2016-03-16 r70336) -- "Very Secure Dishes".

The spatial and temporal variability in community structure was assessed using multidimensional scaling (NMDS). The output of NMDS is a two-dimensional plot with particular configuration of sampling sites at which the distance between ordinated sampling sites correspond to pair-wise similarities in a resemblance matrix. To test whether investigated communities significantly changed their structure after the inundation, a distance-based permutational multivariate analysis of variance (PERMANOVA) was conducted. Samples from different locations in a given flume were combined for this analysis. The input matrix (54 samples (rows), 32 for the analysis of the temporal changes)



and 19 taxa (columns)) for both analyses was based on Bray-Curtis dissimilarities of previously transformed data (fourth root). Community structure was also presented using diversity indices (the total species richness per sample,  $S$ , and abundance,  $N$ ). The Mann-Whitney test was used to compare  $S$  and  $N$  before and after the inundation. This analysis was carried out in SPSS version 15.0.

Indicator value analysis (IndVal) was performed to define positive and negative taxonomical indicators of inundation (Dufrêne & Legendre, 1997). Two groups of samples (before and after inundation) were defined for each flume. A Monte Carlo significance test with 1000 permutations was used to reveal how useful a given species was as an inundation indicator. Taxa with IndVal above 25% and statistical significance ( $p < 0.05$ ) are considered to be significant indicator taxa. This analysis was carried out in SPSS version 15.0.

The oxygen data were analyzed to obtain arithmetic averages of the concentrations with MATLAB, version R2010a (MathWorks) to obtain arithmetic averages of the concentrations.

#### *Hydro-chemical measurements*

Longitudinal profiles of subsurface oxygen concentrations were collected using pre-calibrated O<sub>2</sub> dipping probes (PSt3, PreSens GmbH, Germany) at a separate but comparably vegetated flume. The probes were permanently installed at 10 cm depth along the length of the flume at 1 m increments. Individual measurements were made by connecting individual probes to an oxygen meter (Fibox 3 LCD trace, Presens GmbH, Germany). Measurements were taken, on average, at 5 hours intervals.

## **Results**

#### *Structure of the flume of communities*

We observed 19 taxa of macro- and meiofauna across all flumes (Table 1). Fourteen taxa were macrofauna belonging to the macrobenthos while one mosquito larvae (*Culex pipiens* L., 1758) belongs to a stagnant water neuston (i.e., an organism hanging beneath the water surface, using a hydrophobic structure to attach to the surface film; Lancaster and Downes 2013). The four remaining taxa were classified as meiofauna based on their size (<500 µm).

The macrofaunal communities in all flumes were dominated by non-biting midge larvae (*Chironomus riparius*; Meigen, 1804; Table 1). *C. pipiens* larvae were subdominant in all flumes. Meiofaunal communities were largely comprised of three taxa: cladoceran *Semiocephalus vetulus* (O. F. Müller, 1776), harpacticoids, and Ostracoda. Only one species of a fourth taxa, an undetermined cyclopoid Copepod, was recorded. Flumes vegetated with *S. lacustris* were dominated by Ostracoda. All other flumes were dominated by Harpacticoida (Table 1).

#### *Temporal variability of communities*

Community structure in the flumes differed before and after inundation (Fig. 3: PERMANOVA pseudo- $f = 2.168$ ,  $p = 0.033$ ). Specifically, the specimens density of invertebrates decreased significantly after inundation. This result occurred in all flumes except the *L. vulgaris* flumes in which density did not change significantly (Fig. 2). Two species, *C. pipiens* (IndVal=58.7,  $p=0.023$ ) and *S. vetulus* (IndVal=49.3,  $p=0.049$ ), were most sensitive to the inundation. The abundance of both decreased significantly after inundation.

The effect of inundation on community structure (analyzed both in pooled data set and for individual flumes) in separate flumes across the range of vegetation types showed a similar pattern from all flumes before and after the inundation event. Invertebrate communities in flumes with *Typha angustifolia* (PERMANOVA: pseudo-f=2.342 p=0.044) and *Scirpus lacustris* (PERMANOVA: pseudo-f=2.257, p=0.036) yielded the greatest (quantitative) changes of the communities, manifested as a big drop in the invertebrate densities (Fig. 2A) suggesting that these 2 plant species did not mitigate the effects of inundation. In flumes with *Lysimachia vulgaris* the density of animals in invertebrate community did not significantly change after the inundation (Fig. 3; PERMANOVA: pseudo-f=1.314, p=0.268). Species richness did not change significantly in any of the flumes over the course of the study (Fig. 2).

#### *Spatial variability of communities*

Community structure relative to longitudinal distance within the flume did not significantly differ in any of the flumes (ANCOVA:  $p > 0.05$ ). Pre-flood community structure did not differ significantly between the different helophyte species (NMDS:  $p > 0.05$ ) and the spatial gradient (distance from the flume front) did not influence the variability of community structure during the study (PERMANOVA: pseudo-f=1.182,  $p = 0.240$ ).

#### *Oxygen measurements*

Subsurface oxygen concentrations prior to inundation were consistently below the instrument detection limit of  $0.045 \text{ mg L}^{-1}$ . After inundation, oxygen levels in most locations along the flume increased slightly up to  $0.083 \text{ mg L}^{-1}$  but without any longitudinal pattern.

## **Discussion**

The inundation caused by the AWF resulted in significant decreases in species abundance, but did not significantly decrease the species richness of the hyporheic invertebrate communities, supporting our first hypothesis. Only one helophyte plant species, *Lysimachia vulgaris*, reduced the impact of the inundation on the invertebrates in the flumes, probably due to its dense rhizosphere.

### **Community structure**

Flumes were colonized with pollution-tolerant invertebrates capable of aerial dispersal (i.e., non-biting midges (Chironomidae), marsh flies (Ephydriidae), mayflies (*Centroptilum luteolum*; Müller, 1776), or mosquitos (Culicidae; Lancaster and Downes 2013)) or organisms capable of surviving the physical transport through the WWTP water processing infrastructure during their dormant or active stage (e.g., Tubificidae worms, Copepoda eggs or nauplii, Cladocera ephippium, and Ostracoda) (Lagauzère et al., 2009).

The URL site communities are likely limited by the ability to recruit more species to the flumes. The combination of extremely high levels of nutrient loads and isolation from any major waterbody means that colonization of the flumes may be slow relative to natural (intermittent) river systems (Vander Vorste et al., 2015). Therefore, it must be acknowledged that the URL flumes are not directly comparable to natural or restored rivers in the Mediterranean in terms of community structure. Nevertheless, the URL flumes provide us with a plausible model for functioning of rivers in the region, those with intermittent or low

flow, or those dominated by nutrient and organic matter abundance (Vander Vorste et al., 2015).

In the flumes, there was no significant relationship between the distance from the flume inflow point and the abundance or species richness of the organisms (linear regression analysis,  $p > 0.05$ ). This indicates that factors other than vegetation or stream length within the flume are the primary control of the communities. Spatial changes in oxygen are a possible example of longitudinal controls of community distribution. It is likely, based on longitudinal data from adjacent vegetated flumes, that subsurface oxygen concentrations are at or below the detection limit (0.045 mg/L) in all but the first one meter of the flumes. The consistently anaerobic conditions would also explain why we observed air-breathing species (Culicidae larvae) able to survive under these conditions, and hypoxia tolerant (*Chironomus* larvae) taxa (Brodersen et al. 2008).

**The effects of helophytes on invertebrate reaction to inundation** Differences between the capabilities of the different plant species to reduce inundation may be related to their above and below ground morphology, especially the structure of the root system. *L. vulgaris* is known to be capable of clogging riverine sediments, leading to an acute decrease of the sediment hydraulic conductivity and consequently of hyporheic flow (King County Noxious Weed Control Program, 2010). *L. vulgaris* is also considered to be a dangerous, invasive plant by phytoquarantine authorities at the Pacific Northwest of the United States because of the species ability to invade stream systems (King County Noxious Weed Control Program, 2010). The observed inundation response in the community within the *L. vulgaris* flume could, therefore, be due to such clogging creating refugia, and shielding organisms that would otherwise be washed away by inundation events (Fig. 4 A, B). Thus, we can conclude that the rhizosphere structure of the helophyte is playing an important role in the reduction of inundation effects on the invertebrate communities.

The different species of invertebrates were not equally susceptible to the inundation event. Mosquito larvae (*C. pipiens molestus*) and water flea (*S. vetulus*) were the most affected by the inundation, and were the primary contributors to the drop in abundance after inundation. Neither of these species are benthic, and instead, typically occur in stagnant waters. Larvae of *C. pipiens* are neustonic, living directly under the water surface film and attached to it with a crown of hydrophobic setae on a syphon (the respiration organ at the rear end of the abdomen; Lancaster and Downes 2013). In contrast to other taxa recorded from the flumes, neither *C. pipiens* nor *S. vetulus* have anchoring mechanisms. As such, they are highly susceptible to inundation and are easily carried away by water movement (Lehane 2005; Lancaster and Downes 2013). Both species are likely able to exist in the flumes due to the low flow and large volume of hyporheic water near the bed surface, which essentially creates micro pond-like habitats (Lehane, 2005) in the intergranular water volumes of the coarse gravels. For these two species, unlike the others, the dense root systems and above ground structure of *L. vulgaris* likely reduced the negative effects of AWF, shielding them from being flushed away.

At timescales longer than this flume study, the combination of prolonged wet conditions and *L. vulgaris* might actually lead to a rapid increase in the abundance of *C. pipiens* because the vegetation offers refugia so populations remain high going into the wet period when reproduction success is greatest (Strelková & Halgoš, 2012). Thus, mosquito larvae may actually benefit from certain inundation events in *L. vulgaris* dominated streams.



## Implications for vector management

*Lisymanchia vulgaris* reduction of the inundation negative effects on invertebrates may have implications for public health, as it may favor the development of the mosquito larvae in intermittent rivers. A number of Culicidae species in the Mediterranean are vectors for dangerous infectious diseases (Lehane, 2005). Additional human and livestock nuisance effects of *C. pipiens* may have a significant negative impact on recreational activities in the areas of mosquito mass-emergence (e.g., following an inundation event in a *L. vulgaris* dominated stream system). Although *L. vulgaris* does not selectively promote especially high densities of *C. pipiens* under stable flow conditions (*T. angustifolia* flumes actually have higher percentage of the mosquitos in the communities), it was shown to potentially shield mosquito larvae from hydrological stresses that might otherwise decrease their abundance. Thus, this refugial property of *L. vulgaris* requires further investigation as it may be an additional factor to consider when selecting vegetation types for river restoration projects, especially in the Mediterranean. **Conclusion**

Our experiments show that inundation events have a significant effect on the overall abundance of invertebrates, but not necessarily the species richness. The non-anchored species of mosquito larvae (*C. pipiens molestus*) and water flea (*S. vetulus*) were the species most susceptible and negatively impacted by inundation. The present study also provides evidence that large stature helophytes that interact with both the water column and the hyporheic zone are an important feature offering multiple forms of refugia during inundation events for invertebrates, especially non-anchored species. Such refugia properties of the helophyte are mostly determined by the development of the subsurface rhizosphere. However, it is possible that such vegetational reduction of the inundation negative effects in intermittent rivers from AWF may have its downsides, as *L. vulgaris* seems to promote higher survival of the mosquito larvae which can serve as infectious diseases vectors and create nuisances for humans.

With global climate changes constantly increasing the magnitude of the extreme climatic events, the number of intermittent rivers in the world is increasing (Larned et al., 2010). Prolonged drought events are also leading to the increasing importance of the waste waters as a water source for intermittent rivers (Benotti, Stanford, & Snyder, 2010; Delpla, Jung, Baures, Clement, & Thomas, 2009). The present study thus presents an additional interest in the water resources management context, as it improves our understanding of intermittent rivers under high wastewater loads.

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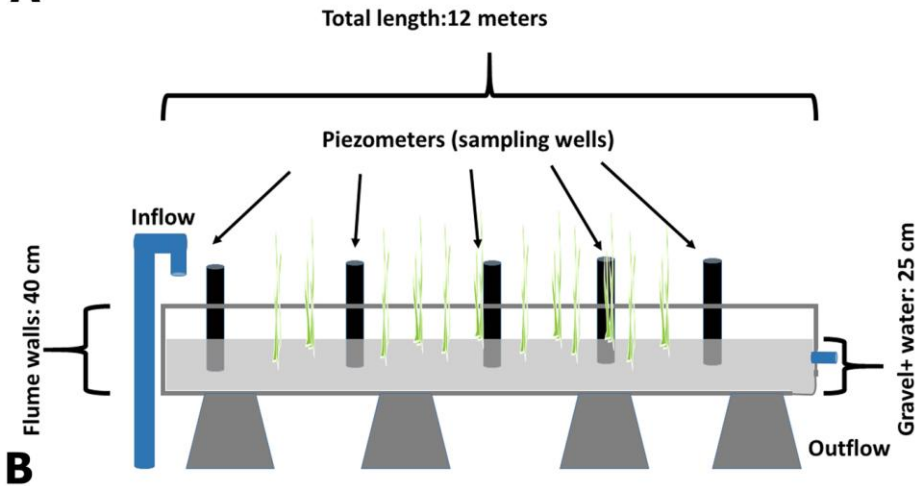
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**A**



**B**

Fig.1 A) View of the URL facility showing control and vegetated flumes used in this study (photo courtesy of Dr. P. Blaen). B) Longitudinal schematic of a flume showing dimensions of flume and the placement of the sampling piezometers.

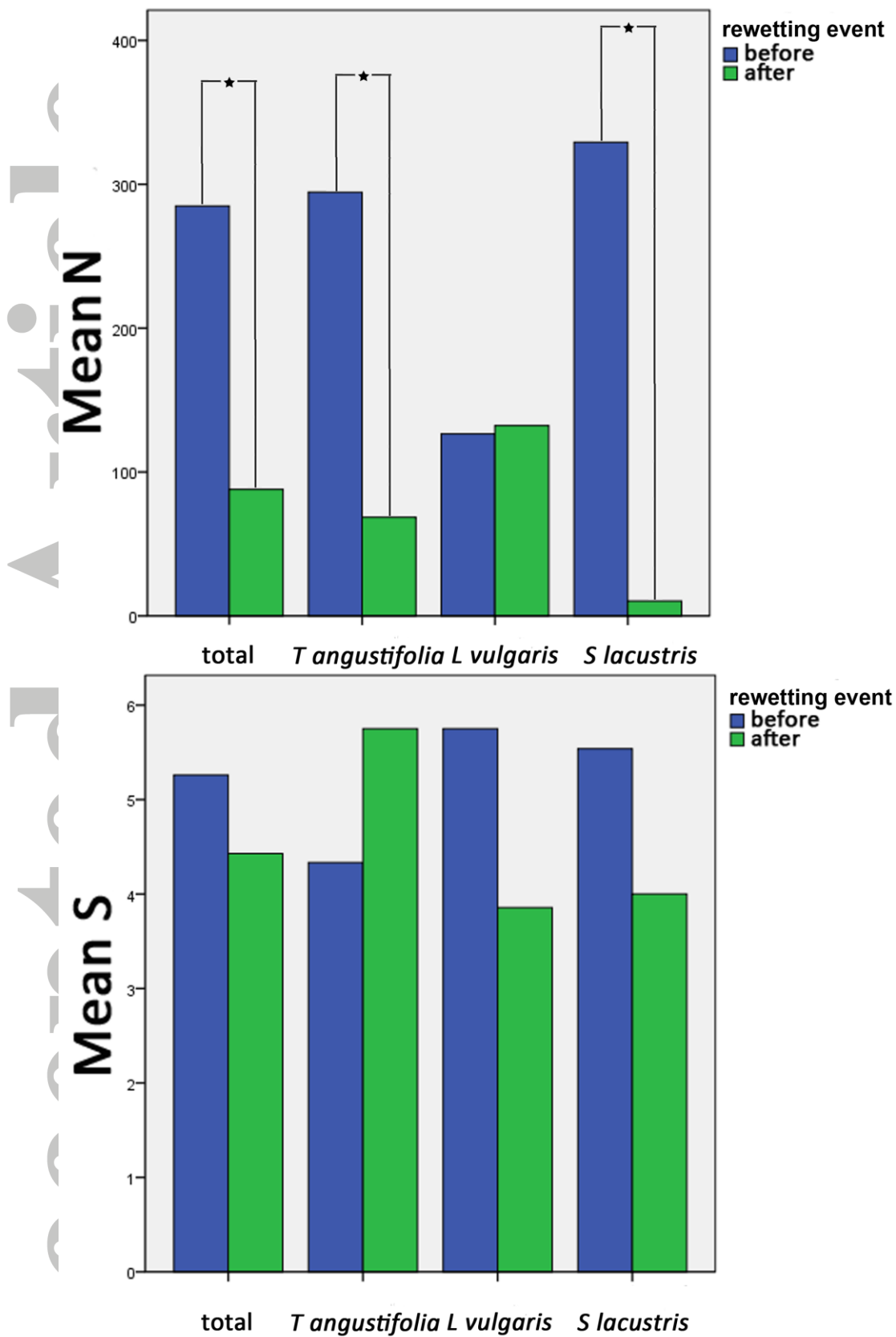


Fig. 2. Impact of inundation event on mean diversity  $S$ , and abundance  $N$  of the invertebrate communities in the flumes. A star indicates significance.



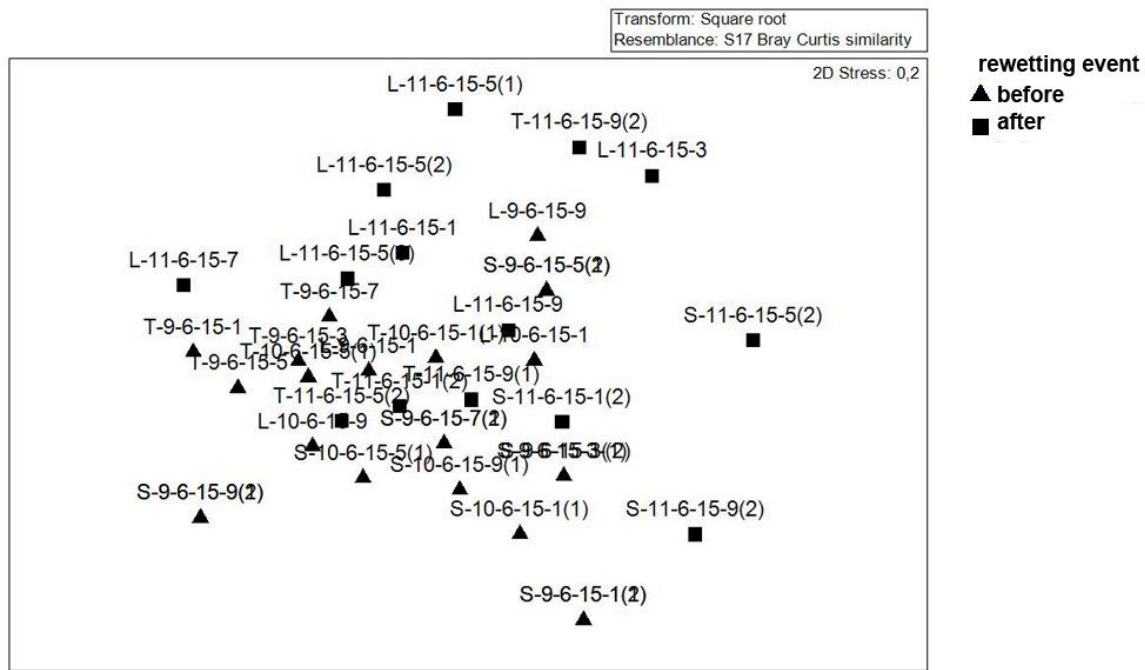


Fig. 3. NMDS of the communities structure before (triangles) and after inundation (squares). S-marked points are standing for *S. lacustris* flumes, L for *L. vulgaris* flumes and T for *T. angustifolia* flumes. Numbers denote the number of the sampling piezometers (see methods).

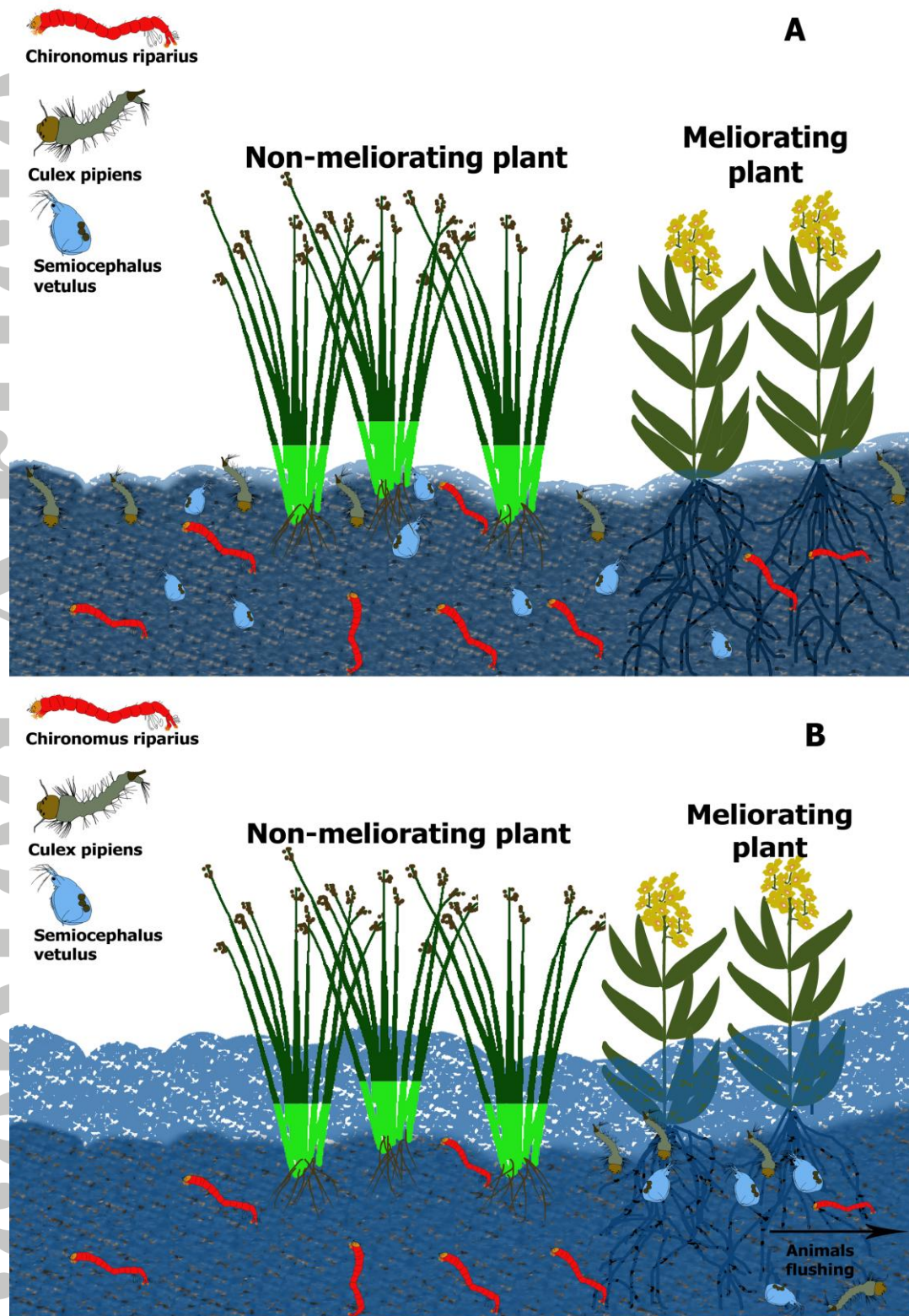


Fig. 4. Conceptual model of URL flumes' community structure A) before and B) after the inundation event. Plants reducing ("meliorating", right) and not affecting (left) the impact of inundation on vulnerable (predominantly non-benthic) members of communities. Hypothetical situation of the both type of helophytes coexisting in the same flume is suggested. It was not a case in the actual set-up.

Table 1. Abundance of macro- and meiofauna in examined URL flumes, before and after inundation categorized by helophyte species. Numbers are percentages of total abundance.

Taxa	<i>Typha angustifolia</i>		<i>Lysimachia vulgaris</i>		<i>Scirpus lacustris</i>	
	before	after	before	after	before	after
Tubificidae	0	1.5	0.2	6.1	0	1.3
Physidae	0.2	0	0.1	0	0	0
<i>Proasselus</i>	0.3	0	1.2	7.6	0.1	0
<i>Hydracarina</i>	0	0	0	0	0.1	0
<i>Centropitulum luteolum</i>	0	0	0	0	0	0
<i>Culex pipiens</i>	26.1	20.2	9.5	4.5	5.8	9
<i>Lbrundinia longipalpis</i>	0	0.4	0	0	0	0
<i>Xenopelopia</i>	0	0	0.1	0	0	0
<i>Chironomus riparius</i>	35.4	23.3	21.1	25.8	41.2	57.7
<i>Cricotopus sp</i>	0.1	1.9	0.2	3	5.1	2.6
<i>Orthocladus sp</i>	0.2	4.2	0	0	0	0
<i>Ceratopogonidae</i>	0	0	0.2	0	0	2.6
<i>Cnestrus sp</i>	0	0	0	0	0	2.6
<i>Scatella sp</i>	0	1.9	0.4	0	0.9	17.9
<i>Setacera</i>	0.1	0	0	0	0	0
<i>Simocephalus vetulus</i>	0.5	3.1	5.3	0	7.5	2.6
Harpacticoida	36.8	42.4	55.6	28.8	3.4	2.6
Copepoda	0	0	0	0	0	0
Ostracoda	0.4	1.1	6	24.2	35.9	1.3